Zootaxa 3994 (1): 094–108
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3994.1.4
http://zoobank.org/urn:lsid:zoobank.org:pub:636D17AA-5A1A-4F67-8952-2E8895CDEC97

A new species of Andean frog of the genus *Bryophryne* from southern Peru (Anura: Craugastoridae) and its phylogenetic position, with notes on the diversity of the genus

JUAN C. CHAPARRO[1,5], JOSÉ M. PADIAL[2], ROBERTO C. GUTIÉRREZ[3] & IGNACIO DE LA RIVA[4]

[1]*Museo de Historia Natural, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Perú. Plaza de armas s/n (paraninfo universitario), Cusco, Perú*

[2]*Section of Amphibians and Reptiles, Carnegie Museum of Natural History 4400 Forbes Avenue, Pittsburgh PA 15213*

[3]*Museo de Historia Natural, Universidad Nacional de San Agustín, Colección de Herpetología, Arequipa, Perú*

[4]*Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales, CSIC. C/ José Gutiérrez Abascal 2, 28006 Madrid, Spain*

[5]*Corresponding author. E-mail: jchaparroauza@yahoo.com*

Abstract

We describe a new species of terrestrial frog of the genus *Bryophryne* (Anura: Craugastoridae) from the wet puna and elfin forests of the Amazonian versant of the Andes. The new species seems to be restricted to high altitude environments at elevations between 3506–3651 m in the area now protected by Megantoni National Sanctuary and Manu National Park (Distrito de Echarate, Provincia La Convención, Departamento Cusco, Peru). The new species is characterized by lacking vomerine processes of vomers, by having tympanic annulus and tympanic membrane not evident through the skin, smooth dorsal skin with scattered warts, conspicuous dorsolateral, middorsal, and occipital folds, warty flanks, areolate skin on ventral surfaces of the body, and by lacking finger and toe fringes and basal web on feet. In life, specimens have bright and highly variable dorsal coloration that ranges from olive-green to red with variable combinations of red or orange marks (red or orange in the green form and olive-green in the red form). Molecular phylogenetic analyses of mitochondrial and nuclear DNA place the new species within the genus *Bryophryne* and as sister group of *B. cophites*. *Bryophryne bustamantei*, also sequenced for this study, is found as the sister group of the clade formed by *B. cophites* and the new species. *Bryophryne* is found as sister group of *Psychrophrynella* in maximum likelihood analyses and as the sister group of a large clade of holoadenines in parsimony analyses. The genus *Bryophryne* now contains nine species, all of them distributed along the Cordillera Oriental of the Peruvian Andes, southeast of the Apurimac River valley.

Key words: Andes, Brachycephaloidea, wet puna, *Psychrophrynella*, *Phrynopus*, taxonomy, terraranas

Resumen

Describimos una nueva especie de rana terrestre del género *Bryophryne* de la puna húmeda y ceja de montaña de la vertiente amazónica de los Andes. La nueva especie habita ambientes de altura entre 3506–3651 m en una zona protegida por el Santuario Nacional Megantoni y el Parque Nacional Manu (Distrito Echarate, Provincia La Convención, Departamento Cusco, Perú). Se caracteriza por carecer de procesos vomerianos y membrana y anillo timpánico visibles a través de la piel, y por poseer una piel dorsal lisa con verrugas dispersas, pliegues dorsolaterales, occipitales y medio dorsales conspicuos, flancos verrugosos, por tener la piel de las zonas ventrales del cuerpo cubiertas de verrugas en aureola, y por carecer de membrana basal y quillas en los dedos. En vida el color del dorso es principalmente verde oliva o rojo, con diferentes combinaciones de rojo y naranja en el morfotipo verde, y de verde en el morfotipo rojo. Los análisis filogenéticos de genes nucleares y mitocondriales indican que la nueva especie es parte del género *Bryophryne*, y que es la especie hermana de *B. cophites*. Además, *Bryophryne* es inferido como el grupo hermano de *Psycophrynella* en análisis de maximum likelihood, mientras que los análisis de parsimonia infieren que este género es el grupo hermano de un diverso clado de especies de Holoadeninae. El género *Bryophryne* incluye ahora nueve especies, todas ellas distribuidas al sur del valle del río Apurimac, en la puna húmeda y bosques de ceja de la Cordillera Oriental de Perú.

Palabras clave: Andes, Brachycephaloidea, filogenética, puna húmeda, *Psychrophrynella*, *Phrynopus*, taxonomía, terraranas

 Accepted by M. Vences: 23 Jun. 2015; published: 30 Jul. 2015

Introduction

Bryophryne was one of the genera recognized by Hedges *et al.* (2008) to amend the polyphyly of the genus *Phrynopus*—most former species of *Phrynopus* are now placed in *Bryophryne*, *Hypodactylus*, *Lynchius*, *Oreobates*, and *Psychrophrynella*, within Holoadeninae (Padial *et al.* 2014; Frost 2015). *Phrynopus cophites* Lynch, 1975, type species of *Bryophryne*, was found to be the closest relative of a clade containing *Barycholos* and *Noblella lochites* (Hedges *et al.* 2008) and another species of *Phrynopus*, *P. bustamantei* (Chaparro *et al.* 2007), was also transferred by Hedges *et al.* (2008) to *Bryophryne* based on morphological resemblance and distribution. Morphological characters proposed to diagnose *Bryophryne*, *Psychrophrynella*, and *Phrynopus* are, nonetheless, basically the same (Hedges *et al.* 2008; Duellman and Lehr, 2009), and *Bryophryne* and *Psychrophrynella* have adjacent distributions in the high Andes, which difficult generic assignment of new species. However, the recent discovery of several new species assigned to *Bryophryne* (Lehr & Catenazzi, 2008, 2009, 2010) suggest that this genus is highly diverse and that all its species are endemic to the Cordillera Oriental of the Andes in southern Peru, east and south of the Apurimac River valley (Lehr & Catenazzi, 2008, 2009, 2010). Herein we present the discovery of another new species of *Bryophryne* from the Amazonian slopes of the Cordillera Oriental of the Andes in southern Peru and assess its phylogenetic position and the monophyly of *Bryophryne* using sequences of mitochondrial and nuclear genes.

Materials and methods

Morphology. Specimens were fixed in 10% formalin and preserved in 70% ethanol. For qualitative and quantitative morphological characters we follow Duellman and Lehr (2009). Only two measurements were omitted, eyelid width and interorbital distance, because they involve soft and often deformed structures difficult to measure with precision (De la Riva 2007). Abbreviations are as follows: SVL (snout-vent length), TL (tibia length), FL (foot length, distance from posterior margin of inner metatarsal tubercle to tip of fourth toe), HL (head length, from posterior margin of jaw to tip of snout), HW (head width, maximum width of head), ED (eye diameter, horizontal), IND (internarial distance), E-N (eye-nostril distance, straight line distance between anterior corner of orbital opening and posterior margin of nostril). Measurements were taken with dial calipers to the nearest 0.1 mm. Specimens were dissected to determine sex and maturity. Museum acronyms are: Museo de Historia Natural, Universidad Nacional de San Antonio Abad, Cusco, Perú (MHNC); and Museo de Historia Natural, Universidad Nacional de San Agustín, Arequipa, Perú (MUSA). Comparisons of diagnostic characters are based on species descriptions in the literature (Lehr and Catenazzi, 2008, 2009, 2010) and the examination of the following type specimens:

Bryophryne bustamantei: Peru: Cusco: Canchayoc: near Abra de Málaga, MHNC 6018 (holotype) 6015–6017, 6019 (paratypes).

Bryophryne cophites: Peru: Cusco: South Slope of Abra Acjanacu, 14 km NNE, Paucartambo, KU 138884 (holotype).

Taxon and character sampling. Phylogenetic analyses in this study employ all DNA sequences of *Bryophryne* available in GenBank as of May 1, 2015. Non-coding mtDNA genes include rRNA genes of the heavy strand transcription unit 1 fragment (12S, 16S and the intervening tRNAvaline, and tRNAleucine segments). Protein-coding mtDNA genes include cytochrome c oxidase subunit I (COI); nuclear protein-coding genes include exon 2 of the cellular myelocytomatosis gene (c-myc), propiomelanocortin A (POMC), recombination-activating protein 1 (RAG1), and tyrosinase precursor (Tyr). We produced 22 new gene sequences of these loci (GenBank accession numbers are: KT276275–KT276296; voucher information for new sequences is provided in Table 1). Genomic DNA was extracted from ethanol-preserved tissues. Extraction, amplification, sequencing, and editing protocols follow those of Padial *et al*. (2012). Primers are listed in Table 2.

TABLE 1. Information of vouchers of *Bryophryne* sequenced for this study (geographical coordinates are listed in the Holotype and Paratype sections).

Species	Tissue library	Voucher	Locality
B. bakersfield	MNCN-DNA20992	MHNC6022	Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Tambo Inca
B. bakersfield	MNCN-DNA20993	MHNC6023	Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Tambo Inca
B. bakersfield	MNCN-DNA21319	MHNC6007	Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Tambo Inca
B. bakersfield	MNCN-DNA21320	MHNC6009	Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Cabecera Timpia
B. bakersfield	MNCN-DNA21322	MHNC5999	Peru: Departamento Cusco: Provincia La Convención: Distrito Echarate: Cabecera Timpia
B. bustamantei	MNCN-DNA21338	MHNC6016	Peru: Departmento Cusco: Provincia La Convención: Distrito de Huayopata: Canchayoc, near Abra de Málaga
B. bustamantei	MNCN-DNA21340	MHNC6019	Peru: Departmento Cusco: Provincia La Convención: Distrito de Huayopata: Canchayoc, near Abra de Málaga

TABLE 2. Sequence primers used in this study.

Locus	Primer name and priming region (5'-3')	Source
16S	16Sar: CGCCTGTTTATCAAAAACAT 16Sbr: CCGGTCTGAACTCAGATCACGT	Hillis *et al.* (1996)
16S	16L19: AATACCTAACGAACTTAGCGATAGCTGGTT 16H24: TACCTTCGCACGGTTAGKRTACCGCGGCCGTT	Hedges *et al.* (2008)
16S	16S-JMP-F: CATGGTAAGTRTACCGGAAGGTG 16S-JMP-R: ACCAGCTATDACTAAGTTCG	This study
12S-tRNA$_{phe}$	12S-t-Phe-frog: ATAGCRCTGAARAYGCTRAGATG 12S-frogRa: TCRATTRYAGGACAGGCTCCTCTAG	Wiens *et al.* (2005)
12S- tRNA$_{val}$	12S-t-Val-frog: TGTAAGCGARAGGCTTTKGTTAAGCT 12S-frogFa: CAAACTRGGATTAGATACCCYACTATG	Wiens *et al.* (2005)
cmyc	cmyc1U: GAGGACATCTGGAARAARTT cmyc3L: GTCTTCCTCTTGTCRTTCTCYTC	Crawford (2003)
COI	AnF1: ACHAAYCAYAAAGAYATYGG AnR1: CCRAARAATCARAADARRTGTTG	Jungfer et al. (2013)
POMC	POMC-1: AATGTATYAAAGMMTGCAAGATGGWCCT POMC-2: TAYTGRCCCTTYTTGTGGGCRTT	Wiens *et al.* (2005)
RAG1	R182: GCCATAACTGCTGGAGCATYAT R270: AGYAGATGTTGCCTGGGTCTTC	Heinicke *et al.* (2007)
TYR	Tyr1C: GGCAGAGGAWCRTGCCAAGATGT Tyr1G: TGCTGGGCRTCTCTCCARTCCCA	Bossuyt & Milinkovitch (2000)

Outgroup sequences were downloaded from GenBank (Table 3). Outgroup selection was guided by previous phylogenetic analyses that place *Bryophryne* with other species of Holoadeninae (Hedges *et al.* 2008; Padial *et al.* 2009; Pyron & Wiens 2011; Padial *et al.* 2014) in the family Craugastoridae (Pyron & Wiens 2011; Padial *et al.* 2014). We sampled 1–4 species per genus of Holoadeninae depending on availability and completeness of available gene sequences. Sequences of the distantly related *Haddadus binotatus* were used to root trees.

Phylogenetic analyses. Parsimony analyses.— Sequences were first aligned in MAFFT (Katoh *et al.* 2005) online version 7, and partitioned into fragments of equal length separated by conserved regions with no gaps and few or none nucleotide substitutions. This strategy generated putatively homologous fragments where length

variation among DNA sequences was only due to insertions and/or deletions of nucleotides, which is a requisite for tree-alignment in POY (Wheeler *et al.* 2006). After the removal of gaps implied by MAFFT from sequence fragments, tree-alignment of unaligned sequences was performed under parsimony with equal weights for all classes of transformations including indels. Tree searches were conducted in POY 5.1.1 (Wheeler *et al.* 2015) under direct optimization (DO, i.e. tree-alignment, Wheeler, 1996; Wheeler *et al.* 2006) using the command "search", which implements a driven search composed of random addition sequence Wagner builds, subtree pruning and regrafting and tree bisection and reconnection branch swapping, parsimony ratcheting, and tree fusing (see Goloboff, 1996, 1999), running consecutive rounds of searches within a specified run-time, storing the shortest trees of each independent run and performing a final round of tree fusing on the pooled trees. The optimal tree found during driven searches was swapped using iterative pass optimization (Wheeler, 2003). Tree searches were carried out using the American Museum of Natural History's high performance computing cluster ENYO. We calculated Goodman-Bremer (GB) values for each supported clade in TNT (Goloboff *et al.* 2008) using the optimal tree-alignment matrix and the parameters specified in the bremer.run macro. We also calculated parsimony jackknife frequencies (Farris *et al.* 1996) for each supported clade by resampling the tree-alignment matrix. Given that the tree-alignment matrix is derived from the optimal tree, the resulting clade frequencies are expected to be higher than would be obtained from matrices aligned according to different guide trees (*e.g.,* a UPGMA tree as in MAFFT). We calculated jackknife frequencies from 1000 pseudoreplicates searches using driven searches (see below), gaps treated as fifth state, and removal probability of 0.36 ($\approx e^{-1}$), which purportedly renders jackknife and bootstrap values comparable (Farris *et al.* 1996).

Maximum likelihood analyses.—The MAFFT alignment edited for POY was used for maximum likelihood analyses. We used PartitionFinder V1.1.1 (Lanfear *et al.* 2012) to select the optimal partition scheme and substitution models for our dataset under the Akaike Information Criterion (AIC), the Bayesian Information Criterion (BIC), and the Corrected Akaike Information Criterion (cAIC). Compared partition schemes were: (1) all data combined, (2) a 2-partition, mtDNA/nuDNA, scheme, (3) by locus (each partition corresponding to individual loci mentioned above), and (4), by locus and codon position (for protein coding genes). Adaptive tree searches and 1000 bootstrap replicates were performed using random addition sequence replicates using GARLI 2.0 (Zwickl 2006) through the GARLI Web Server (Bazinet *et al.* 2014). Gaps were treated as absence of evidence (i.e., missing data).

Results

Descriptive data on the external morphology, including characters evidencing divergence of the new species (i.e., unique character states) from other species in the genus are listed in the diagnosis. Other traits are described in the holotype description and variation sections below.

The tree-alignment from POY resulted in 5936 character columns for 37 terminals while the MAFFT alignment for the same data resulted in 5310 characters. Tree searches in POY rendered two equally optimal trees of 9129 character transformations, which after iterative pass optimization resulted in a single optimal tree of 9074 transformations and identical relationships (Fig. 1A). The best maximum likelihood tree had log likelihood = -43164.04 (Fig. 1B). Both analyses inferred the new species as monophyletic, as part of *Bryophryne*, and as sister to *B. cophites*. *Bryophryne bustamantei*, a species removed from *Phrynopus* and transferred to *Bryophryne* by Hedges *et al.* (2008) on the basis of external morphology and distribution is corroborated as part of *Bryophryne*. However, parsimony and maximum likelihood analyses differ with respect to the position of *Bryophryne* within Holoadeninae. Parsimony analyses place *Bryophryne* as the sister group of a clade formed by species of *Euparkerella*, *Holoaden*, *Hypodactylus*, *Lynchius, Oreobates*, *Phrynopus*, and *Psychrophrynella*. Maximum likelihood places *Bryophryne* as the sister group of *Psychrophrynella*, and this clade is sister to one that includes *Barycholos*, *Holoaden*, *Euparkerella*, "*Eleutherodactylus" bilineatus*, and *Noblella*.

TABLE 3. GenBank codes for sequences of outgroups used in this study.

	12S tRNA$_{val}$ tRNA$_{leu}$	16S	cmyc	COI	POMC	RAG1	TYR
Barycholos pulcher	EU186727	EU186709				EU186744	EU186765
Barycholos ternetzi		DQ283094		JX298356	JX298136		DQ282921
Bryophryne cophites	EF493537	EF493537				EF493423	EF493508
Eleutherodactylus bilineatus	JX267393	JX267324				JX267556	JX267691
Euparkerella brasiliensis	JX267390	JX267468				JX267545	JX267682
Haddadus binotatus	EF493361	EF493361	GQ345147	JX298361	GQ345259	EF493397	DQ282918
Holoaden bradei	EF493378	EF493366		JX298358	JX298138	EF493449	EU186779
Holoaden luederwaldti	EU186728	EU186710				EU186747	EU186768
Hypodactylus brunneus	EF493357	EF493357	GQ345151		GQ345264	EF493422	EF493484
Hypodactylus dolops	EF493394	EF493394				EF493414	EF493483
Hypodactylus elassodiscus	EF493358	EF493358					
Hypodactylus peraccai	EF493710	EF493710				EF493420	EF493485
Lynchius flavomaculatus	EU186667	EU186667				EU186745	EU186766
Lynchius nebulanastes	EU186704	EU186704	AY819320		AY819154		
Lynchius parkeri	EU186705	EU186705					
Lynchius simmonsi	JF809940	JF810004				JF809915	JF809894
Noblella heyeri	JX267463	JX267541					
Noblella lochites	EU186699	EU186699				EU186756	EU186777
Noblella myermecoides	JX267464	JX267542					
Oreobates ayacucho	JF809933	JF809970				JF809912	JF809890
Oreobates cruralis	EU186666	EU186666				EU186743	EU186764
Oreobates quixensis	EF493828	EF493662	AY819178	JX298360	AY819093		QZ31186
Phrynopus auriculatus	EF493708	EF493708					
Phrynopus bracki	EF493709	EF493709	GQ345150		GQ345263	EF493421	EF493507
Phrynopus tribulosus	EU186725	EU186725					
Psychrophrynella guillei	AY843720	AY843720					DQ282995
Psychrophrynella iatamasi	AM039712	AM039644		JX298362			
Psychrophrynella saltator	AM039710	AM039642					
Psychrophrynella usurpator	EF493714	EF493714				EU186762	EU186780
Psychrophrynella wettsteini	EU186696	EU186696	GQ345153		GQ345266	EU186755	EU186776

.

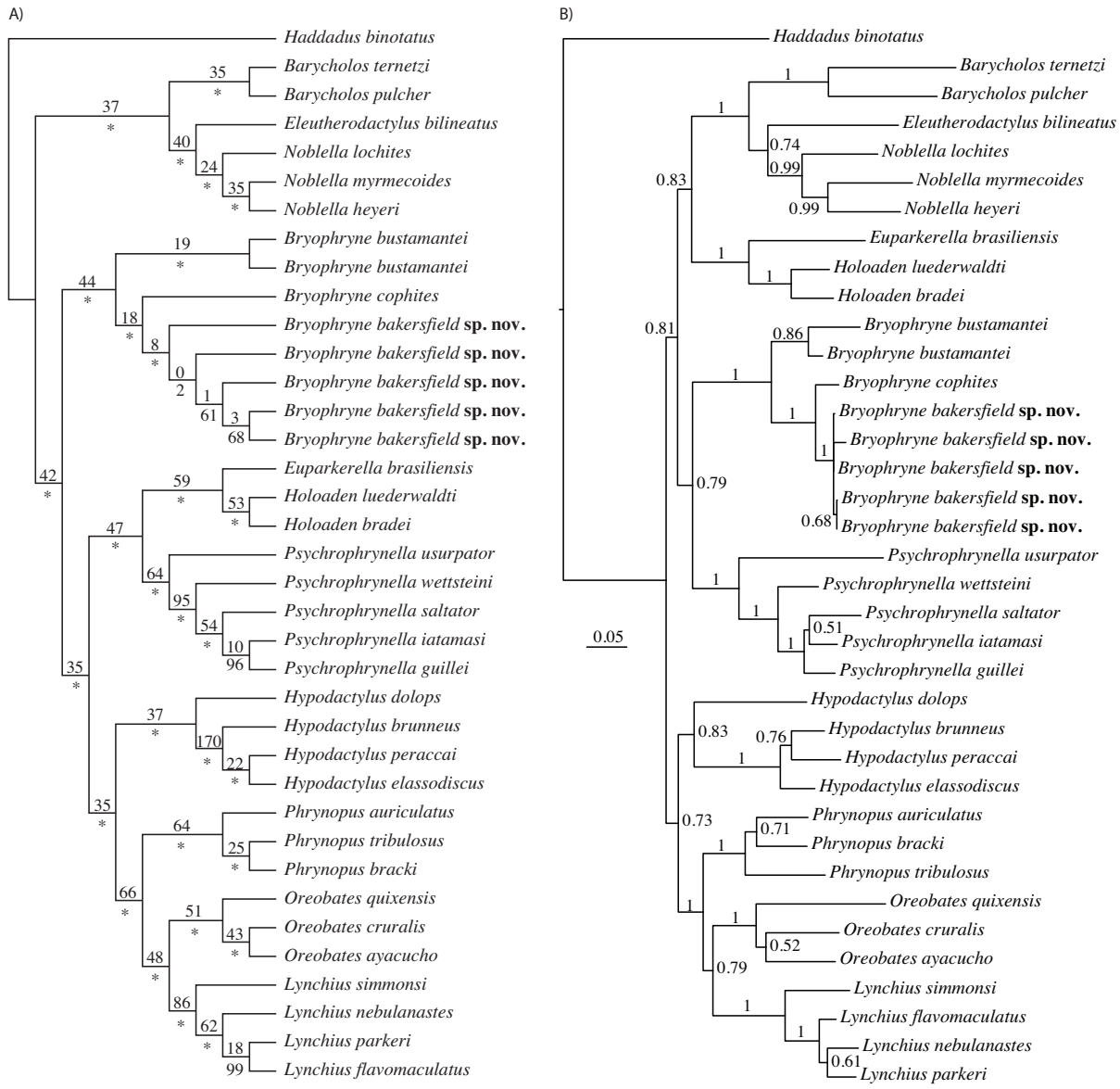

FIGURE 1. Phylogenetic trees resulting from the analysis of a dataset of 5310 aligned bp (MAFFT) and 5936 bp (POY) composed by the mitochondrial genes 12S, 16S, and the intervening $tRNA_{valine}$, and $tRNA_{leucine}$ segments, a fragment of the mtDNA gene COI, and fragments of the nuclear protein-coding genes c-myc (exon 2), POMC, RAG1, and Tyr. (A) Maximum parsimony optimal tree of 9074 transformations depicting the relationships of species of *Bryophryne* and other genera of Holodeninae. Numbers above branches indicate Goodman-Bremer values, and those under branches are Jackknife proportions (asterisks represent Jackknife values of 100%). (B) Maximum likelihood optimal tree (log likelihood = -43164.04) and bootstrap node values.

Bryophryne bakersfield sp. nov.

(Figs. 2–4)

Holotype. MHNC 7972, field code RGP 2728, an adult female (Fig. 2) from Roquerío de Lorohuachana, 3620 m a.s.l. (12° 29' 43.8"S, 72° 04' 35.9"W, Fig. 5), Distrito de Echarate, Provincia de La Convención, Departamento Cusco, Peru, collected by R. Gutiérrez on 17 June 2008.

Paratypes. All from localities in the Distrito de Echarate, Provincia La Convención, Departamento Cusco, Peru: MHNC 7973, MUSA 2362 (adult females), MUSA 2360, 2363–2365 (adult males), MUSA 2361 (subadult male), from the type locality, collected by R. Gutiérrez, H. Zamora, and D. Huaman, on 13–18 June 2008; MHNC

5993, 6001 (adult females), MHNC 5996–5997, 6000 (adult males) from 2.7 km NW, Roquerío de Lorohuachana, 3560 m a.s.l. (12° 29' 11.55"S, 72° 06' 03.45"W), collected by R. Gutiérrez on 17 June 2008; MHNC 6006, 6014 and MNCN 43707 (adult females), MHNC 6007, 6010, 6012, 6022–6023 (adult males), MHNC 6008, 6013 (subadult females), from Tambo Inca, 3651 m a.s.l. (12° 29' 09.74"S, 72° 04' 04.66"W), collected by A. Pari, C. Condori, and W. L. Delgado, on 12–21 April 2007; MHNC 7975, MUSA 2357 (adult females) and MHNC 7974 (subadult female) from Tres Claveles, 8.7 km SW, Roquerío de Lorohuachana, 3393 m a.s.l. (12° 32' 43.9" S, 72° 08' 22.7" W), collected by R. Gutiérrez on 12 June 2008; MUSA 2358 (adult male) and MUSA 2359 (subadult female) from Yanacocha lake surroundings, 4.3 km SW of Roquerío de Lorohuachana, 3506 m a.s.l. (12° 31' 36.8'' S, 72° 05' 59.5" W), collected by R. Gutiérrez on 13 June 2008; MUSA 2367–2368 from Cajoniyoc Pass surroundings, 3.0 km SW of Roquerío de Lorohuachana, 3604 m a.s.l. (12° 28' 8.10'' S, 72° 04' 12.77" W), collected by R. Gutiérrez on 19 June 2008; MHNC 5999 (adult male) and MHNC 6002 (subadult female), from Cabecera Timpia, 3579 m a.s.l. (12° 29' 11.55"S, 72° 06' 03.45"W), Departamento Cusco, Peru, collected by A. Pari, C. Condori, and W. L. Delgado on 19 April 2007.

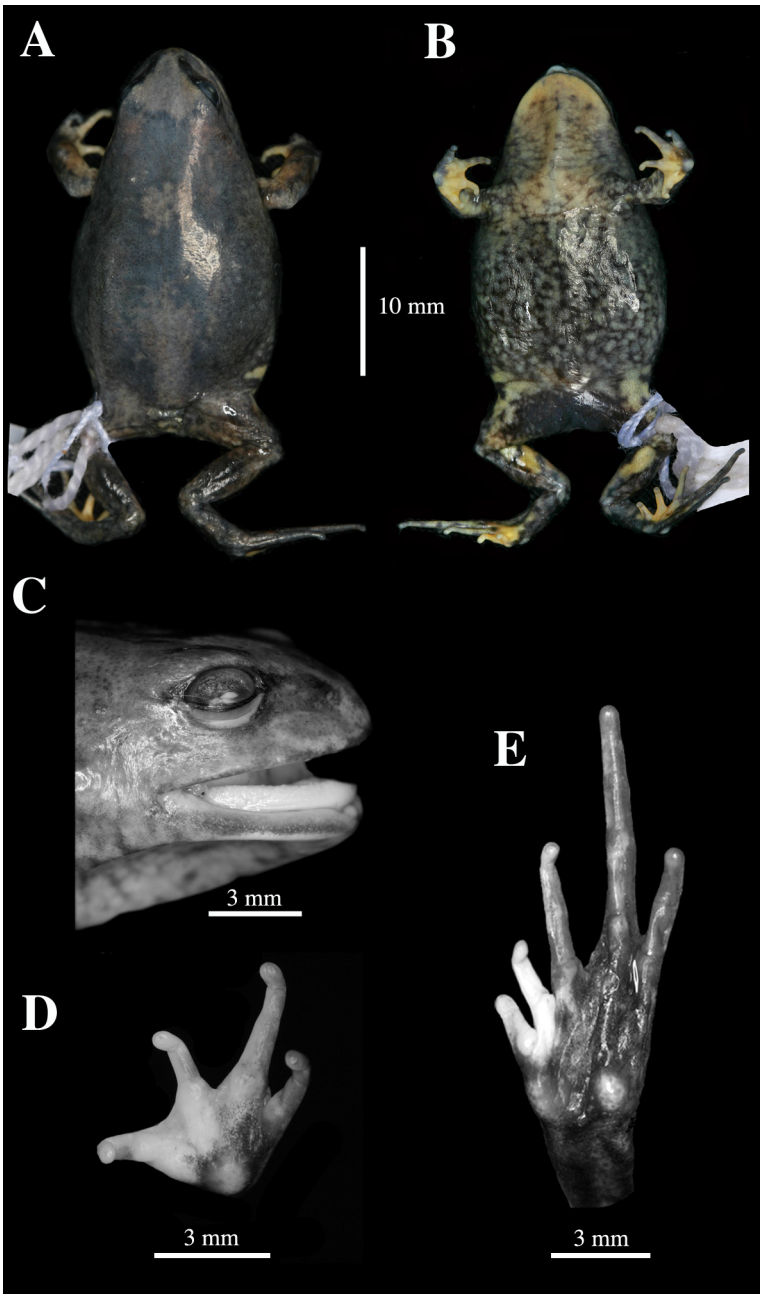

FIGURE 2. Dorsal (A), and ventral (B) views of the body, lateral view of head (C), hand (D), and foot (E) of the adult female holotype (MHNC 7972) of *Bryophryne bakersfield* **sp. nov.**

Diagnosis. The new species is part of *Bryophryne* according to molecular phylogenetic relationships (Fig. 1). *Bryophryne bakersfield* is characterized by: (1) skin on dorsum smooth or rugose with warts; flanks densely warty, with larger warts than those of dorsum; dorsal, dorsolateral, occipital and supratympanic folds prominent (not apparent in preservative); ventral skin areolate, throat and chest areolate but with smaller warts; dorsal and ventral warts covered by minute keratinous spicules; (2) tympanic membrane and tympanic annulus absent; (3) snout short, rounded in dorsal and lateral views; (4) upper eyelid with or without enlarged tubercles, cranial crests absent; (5) dentigerous process of vomers absent; (6) vocal sac small, vocal slits present, nuptial pads absent; (7) Finger I shorter than, or equal to Finger II, tips of digits rounded; (8) fingers lacking lateral fringes; (9) ulnar tubercles absent; (10) heel bearing one or two small low tubercles, tarsus lacking tubercles and folds; (11) plantar surfaces of feet bearing two metatarsal tubercles, the inner slightly larger than the outer; supernumerary plantar tubercles low, weakly defined; (12) toes lacking lateral fringes; webbing absent; Toe III longer than Toe V, tips of digits rounded; (13) dorsal and ventral coloration variable, including red, orange, yellow, brown, green, cream or gray colors; groin and shanks with yellow flash marks; (14) SVL 25.0–31.1 mm in females, 17.3–22.9 mm in males (Table 4).

TABLE 4. Measurements (in mm) and proportions of adult specimens of *Bryophryne bakersfield*. Mean and standard deviation in parenthesis follow ranges.

	Adult females (n=8)	Adult males (n=12)
SVL	25.0–31.1 (27.6±2.1)	17.3–22.9 (20.3±2.1)
TL	8.2–10.0 (9.0±0.5)	6.5–7.9 (7.2±0.5)
FL	9.4–11.9 (10.5±0.8)	7.5–8.9 (8.1±0.5)
HL	7.2–9.0 (7.7±0.5)	5.6–7.1 (6.2±0.5)
HW	8.4–10.0 (9.2±0.5)	6.2–7.7 (7.1±0.5)
ED	2.6–3.2 (2.8±0.2)	1.8–2.6 (2.1±0.3)
IND	2.0–2.3 (2.2±0.1)	1.4–2.1 (1.8±0.2)
END	1.8–2.3 (2.1±0.2)	1.5–2.1 (1.7±0.2)
HL/SVL	0.3–0.3 (0.3±0.0)	0.3–0.3 (0.3±0.0)
HW/SVL	0.3–0.4 (0.3±0.0)	0.3–0.4 (0.4±0.0)
END/ED	0.7–0.9 (0.8±0.1)	0.7–0.9 (0.8±0.1)
TL/SVL	0.3–0.4 (0.3±0.0)	0.3–0.4 (0.4±0.0)
FL/SVL	0.3–0.4 (0.4±0.0)	0.3–0.4 (0.4±0.0)

Bryophryne bakersfield is most similar to *B. bustamantei* (Fig. 6), with which it shares the presence of complete dorsolateral folds, warty flanks, presence of vocal slits, and the absence of tympanic membrane, annulus, and dentigerous process of vomers. The species differ in that the dorsal skin of *B. bustamantei* is coarsely shagreen (densely covered with small granules)—a character most evident in life—whereas the dorsal skin of *B. bakersfield* is smooth and covered with scattered warts and low folds. From the rest of species of *Bryophryne*, *B. bakersfield* differs in the following character states (states of other species in parentheses). From *B. abramalagae* by having vocal slits (absent), lacking ulnar and tarsal tubercles and basal membrane on feet (present), and having Toe III longer than Toe V (equal). From *B. cophites* (Fig. 6), by having warty dorsal skin on smooth background (shagreen), and broad, high and complete dorsolateral folds (short, low, incomplete or absent). From *B. flammiventris* by lacking tympanic membrane (present), a large supratympanic fold (large and conspicuous supra tympanic fold), and a well-developed vocal sac (very large vocal sac). From *B. gymnotis,* by having an areolate belly (smooth), broad, high and complete dorsolateral folds (narrow and discontinuous), warty dorsal skin on smooth background (shagreen), and absence of tympanic membrane and annulus (present). From *B. hanssaueri*, by having warty dorsal skin on smooth background (shagreen), broad, high and complete dorsolateral folds (short and incomplete), absence of ulnar fold (present), absence of finger and toe fringes (present), and smaller size (maximum size 24.6 mm). From *B. nubilosus* (Fig. 6) by having warty dorsal skin on smooth background (shagreen), vocal slits present (absent), ulnar and tarsal tubercles absent (present), and smaller size (maximum SVL 21.9). From *B. zonalis,* by having warty dorsal skin on smooth background (shagreen), broad, high and complete dorsolateral folds (short, low and incomplete), ulnar and tarsal tubercles absent (present), finger and toe fringes

absent (present), basal toe webbing absent (present), vocal slits present (absent), and smaller size (maximum SVL 24.4 mm).

Description of the holotype. Body robust; dorsal skin warty on smooth background, with enlarged warts in occipital region and flanks; ventral skin areolate; dorsolateral folds prominent, from above the ocular region to the level of the sacral region; a pair of dorsolateral folds; pectoral fold present; head wider than long; HW 30% of SVL, HL 25% of SVL; snout short, rounded in dorsal view and in profile; nostrils prominent, closer to snout than to eyes; canthus rostralis concave in dorsal view, rounded in profile; eye-nostril distance 76% of eye length; loreal region slightly concave; cranial crests absent; tympanic membrane and tympanic annulus absent, skin of the tympanic area covered by low, round subconical warts; supratympanic fold small; tongue large, oval; choanae small, rounded, separated by 2 mm; dentigerous processes of vomers absent; limbs moderately short; tips of digits round, not expanded laterally; ulnar tubercle and fold absent; inner palmar tubercle single, round; fingers moderately short, not fringed; subarticular tubercles small, round; supernumerary tubercles small and inconspicuous; first finger shorter than second, relative length of fingers I ≤ II = IV < III; tibia length 32% of SVL; tarsal fold absent; two metatarsal tubercles, inner slightly larger than oval outer; supernumerary tubercles small, poorly defined; subarticular tubercles of toes round; toes lacking basal webbing or lateral fringes; relative length of toes I < II < III = V < IV; foot length 38% of SVL.

In life, the dorsum of the holotype was mostly greenish-brown, the dorsolateral and mid-dorsal folds were dark brown, and the dorsal surfaces of hands and feet were yellowish-orange. The belly was dark brown with cream blotches, and the throat was yellow; the fingers, toes and plantar surfaces were yellowish-orange; the groin and shanks had yellow flash marks; the iris was light metallic blue with black reticulations. In preservative, the dorsum is grey, the venter is dark with cream blotches, and yellowish-orange surfaces turned cream.

Measurements (in mm) of the holotype: SVL, 31.1; HL, 7.9; HW, 9.3; IND, 2.3; E-N, 2.3; ED, 3.0; TL, 10.0; FL, 11.9.

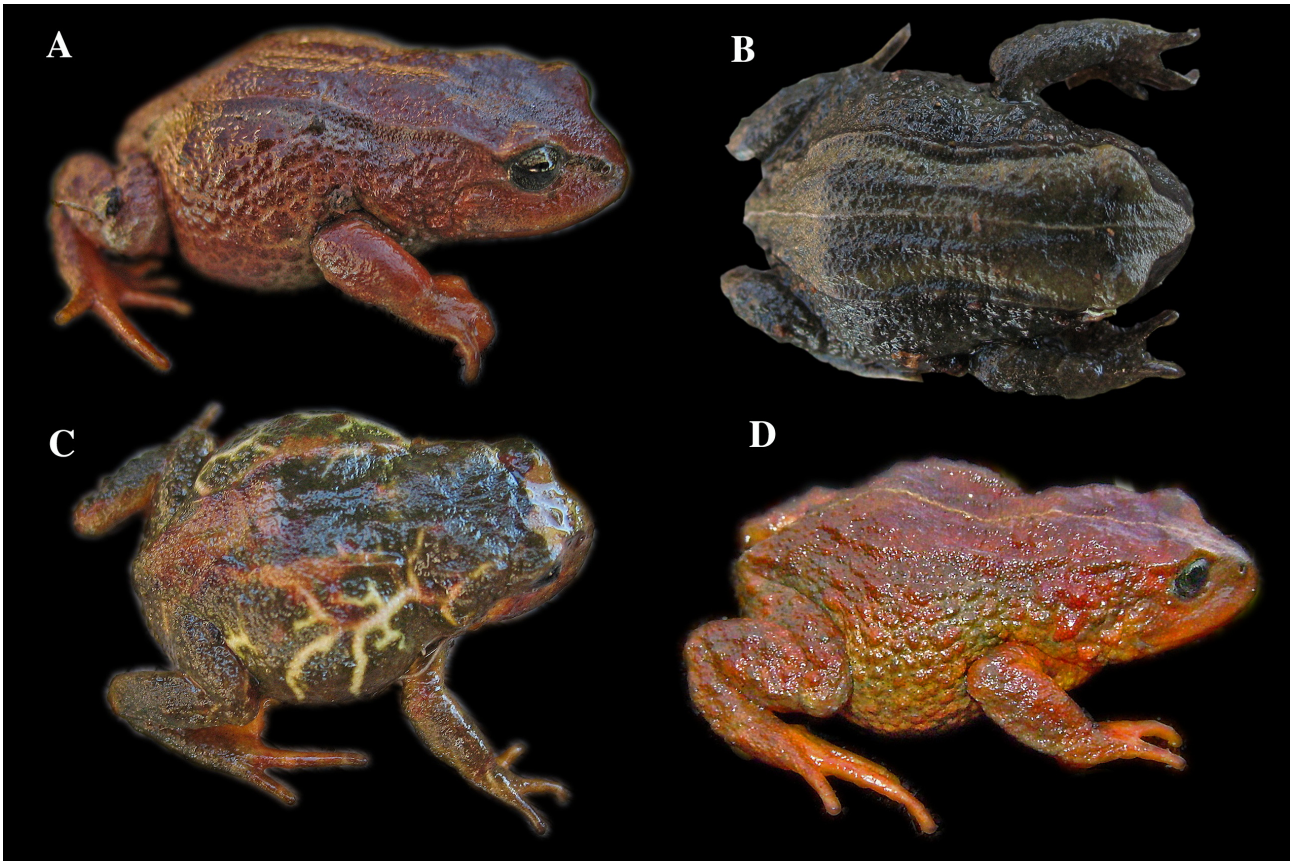

FIGURE 3. Lateral views showing different color patterns and dorsal skin textures of *Bryophryne bakersfield* **sp. nov.** (A) MUSA 2368, SVL=27.2 mm; (B) MHNC 7973, SVL=25.0 mm; (C), MUSA 2367, SVL=24.0 mm; (D) MUSA 2357, SVL=25.6 mm.

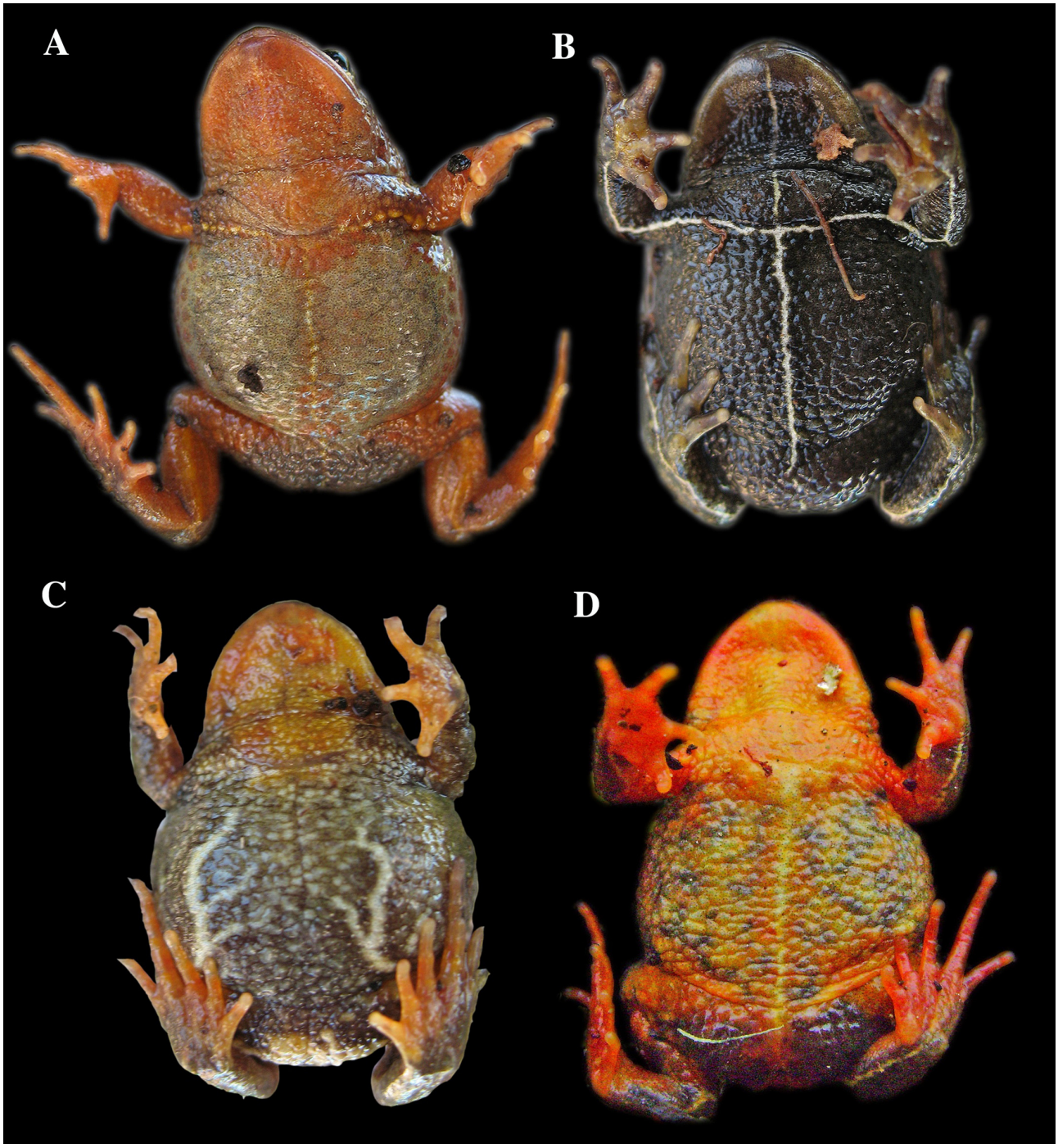

FIGURE 4. Ventral views showing different color patterns and skin texture of *Bryophryne bakersfield*. (A) MUSA 2368, SVL=27.2 mm; (B) MHNC 7973, SVL=25.0 mm; (C), MUSA 2367, SVL=24.0 mm; (D) MUSA 2357, SVL=25.6 mm.

Variation. Males possess vocal sac and slits and are smaller than females, but otherwise are similar in proportions (Table 4). Both males and females are variable in ventral and dorsal coloration and in the degree of development and presence of dorsal, occipital and dorsolateral folds. Dorsal coloration can range from dark red (e.g. MUSA 2368; Fig. 3A), to pale or dark olive-green (MHNC 7973, Fig. 3B), or a mix of red, orange, cream, and green shades (e.g. MUSA 2367 or MUSA 2357). The dorsum and belly of some specimens have sinuous cream stripes (e.g. MUSA 2367, Fig. 3C, and MUSA 2366). A fine middorsal yellow, cream or gray stripe is present in some specimens (e.g. MHNC 7973 and MUSA 2357; Fig. 3B and 3D). Dorsolateral folds are mostly present, and are complete, high, and conspicuous, as in MUSA 2368 and MHNC 797 (Fig. 3A and 3B). Some specimens also have a pair of well-developed middorsal folds internal and parallel to the dorsolateral folds (e.g. MHNC 7973, Fig.

3B, and MUSA 2363), that in some cases are sinuous (e.g. MUSA 2358). The ventral skin texture is always areolate, although the warts are more evident in some specimens than in others (e.g. Fig. 4). The throat and chest have smaller warts, sometimes resembling granules (e.g. MUSA 2368, Fig. 4A). The ventral coloration is highly variable (Fig. 4), ranging from dark olive-green (MHNC 7973; Fig. 4B), to pale gray with orange (e.g. MUSA 2368, Fig. 4A), or intense orange and yellow (MUSA 2357; Fig. 4D); a cream or yellow cross-mark is present in some specimens (e.g. MUSA 2368, MHNC 7973, and MUSA 2357; Fig. 4A–B and D); two specimens have sinuous, cream ventral stripes (e.g. MUSA 2367, Fig. 4C, and MUSA 2366).

Etymology. The species epithet, used as a noun in apposition, refers to the city of Bakersfield, in the San Joaquín Valley of California. The name was selected by Javier Bustamante, a Peruvian residing in Bakersfield, who funded Juan C. Chaparro's research in Peru.

Distribution and natural history. *Bryophryne bakersfield* is known from four close localities at elevations of 3506–3651 m a.s.l. on the Amazonian versant of the Cordillera Oriental of the Peruvian Andes, Departamento Cusco, southern Peru (see above). Like other species of *Bryophryne*, *B. bakersfield* inhabits wet puna and the upper limits of elfin forest or "ceja de montaña". Specimens were collected at the end of the rainy season and during the dry season inside clumps of grass and under stones and fallen trees. Males were calling during the day around noon. Other amphibians found in sympatry were *Gastrotheca excubitor* and *Telmatobius* sp.

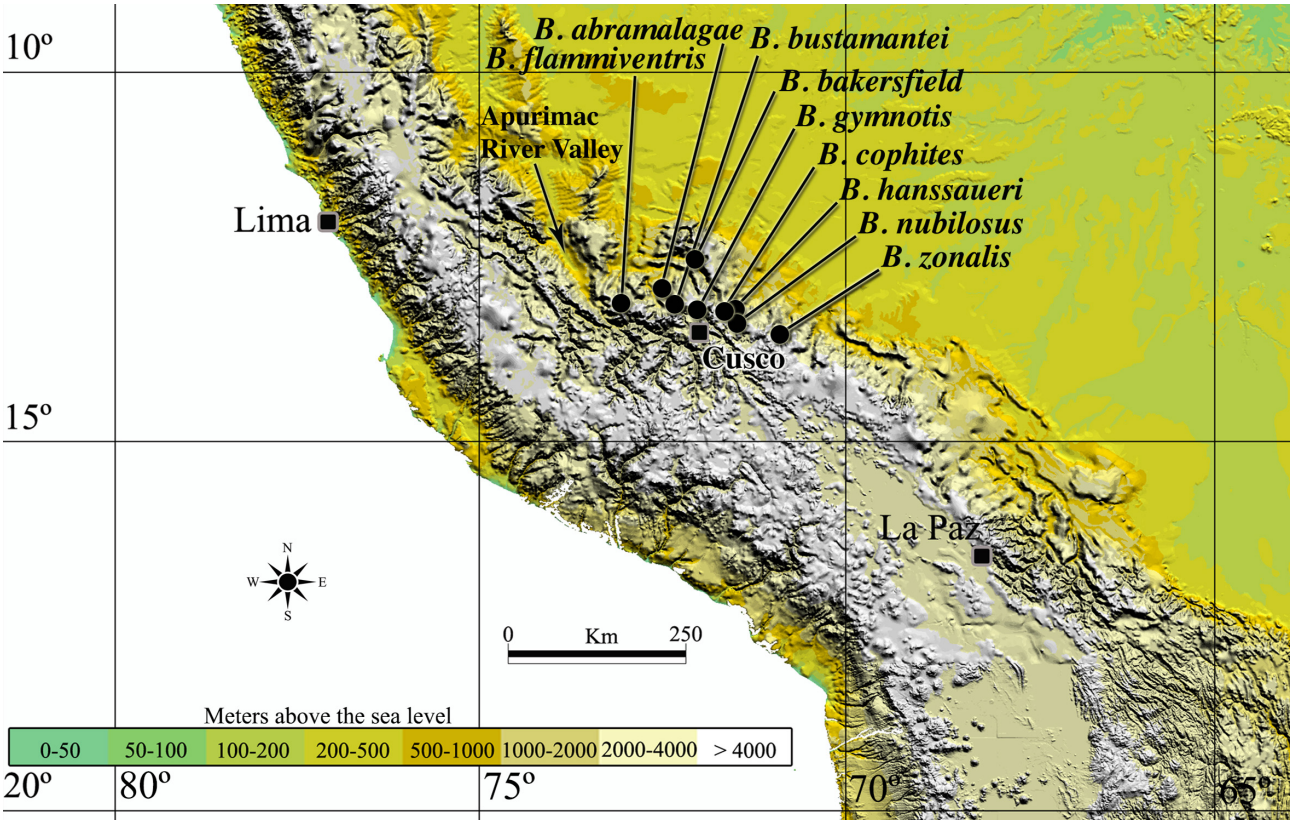

FIGURE 5. Map of the Peruvian Andes, showing the type localities of the species of *Bryophryne*.

Discussion

The non-monophyly of *Phrynopus* Peters (*sensu* Lynch, 1975) was inferred by Lehr *et al.* (2005) and Heinicke *et al.* (2007) on the basis of molecular data, and Hedges *et al.'s* (2008) comprehensive revision of the systematics of terraranas (Brachycephaloidea) led to the division of *Phrynopus* into six genera. Hedges *et al.* (2008) erected *Bryophryne* because *Phrynopus cophites* (Lynch, 1975) was found as the sister group of *Noblella* + *Barycholos*, or, alternatively, as the sister group of a clade that they named as the genus *Psychrophrynella*. Subsequent studies have corroborated *Bryophryne* as part of Holoadeninae and as the sister group of *Holoaden* (Pyron & Wiens 2011), *Euparkerella* + *Holoaden* (Canedo & Haddad 2012) or *Noblella* + *Barycholos* (e.g., Padial *et al.* 2014).

Phylogenetic analyses in this study provide support for the monophyly of *Bryophryne* using three species but are ambiguous with respect to the position of the genus (*Bryophryne* was recovered as either the sister group of *Psychrophrynella* in maximum likelihood or as the sister group of a large clade of holoadenines in parsimony analyses). Nonetheless, taxon sampling in our study was designed only to test the monophyly of *Bryophryne* and to infer the position of *B. bustamantei* and *B. bakersfield*. A rigorous assessment of the position of *Bryophryne* within Holoadeninae would have required a larger taxon sampling and therefore our results should be interpreted with caution.

FIGURE 6. Top: Dorsal and ventral view of an adult male of *Bryophryne bustamantei* (MHNC 6017, SVL=18.5 mm) from near Canchayoc, Departamento Cusco, Peru. Middle: Dorsal and ventral view of an adult female of *B. cophites* from Pantillacocha, Distrito Kosñipata, Provincia Paucartambo, Departamento Cusco, Peru (MHNC 4651, SVL=28.3 mm). Bottom: Dorsal and ventral view of *B. nubilosus* from Esperanza, Distrito Kosñipata, Provincia Paucartambo, Departamento Cusco, Peru (MHNC 4588, SVL=17.3 mm).

Morphological characters purportedly diagnosing *Bryophryne* and other Andean genera of Holoadeninae (*Hypodactylus*, *Lynchius*, *Oreobates*, *Phrynopus*, *Psychrophrynella*) do not always allow unambiguous generic assignation of new high Andean species with *Phrynopus*-like morphologies —i.e., adapted to high Andean grasslands. Most recent allocations of new species to any of these genera have been based on distribution (Lehr & Oróz 2012, Lehr *et al.* 2012, De la Riva & Burrowes 2014, Mamani & Malqui 2014), because the currently known distribution of genera do not overlap (e.g., *Bryophryne* and *Phrynopus*) or only do it in small portions of their distributions (e.g., *Oreobates* and *Phrynopus*). Nonetheless, morphology (Padial *et al.* 2012), and especially molecular data (Padial *et al.* 2012, Teixeira *et al.* 2012, Pereyra *et al.* 2014) have also been important sources of evidence for generic allocation in Holoadeninae. However, the search for putative synapomorphies for *Bryophryne* among external morphological characters has been unsuccessful. Lehr and Catenazzi (2008) discovered the shared absence of tympanum and dentigerous process of vomers in *B. bustamantei*, *B. cophites*, and *B. nubilosus*, (these states are also present in *Phrynopus* and *Psychrophrynella*). More recently, Lehr and Catenazzi (2009, 2010) named two species of *Bryophryne* that present a distinct annulus and tympanic membrane (*B. flammiventris* and *B. gymnotis*). Thus, the generic allocation of six species of *Bryophryne* (*B. abramalagae*, *B. flammiventris*, *B. gymnotis*, *B. hanssaueri*, *B. nubilosus*, and *B. zonalis*), and the monophyly of this taxon, remains to be tested, although geographic proximity in the distribution ranges of species suggests that they may well be part of a monophyletic group. Indeed, all nine species of *Bryophryne* named so far are geographically restricted to the Andean wet puna and elfin forests of the Cordillera Oriental of Departamento Cusco, north and east of the Apurimac river valley, a valley that was proposed by Lehr and Catenazzi (2008, 2009) and Duellman and Lehr (2009) as a biogeographic boundary for the occurrence of *Bryophryne*, northwestward of which only *Phrynopus* would occur. The discovery of *B. bakersfield* and our phylogenetic analysis of the genus *Bryophryne* provides some support for that hypothesis. However, some high Andean species of *Oreobates* or even *Pristimantis* with similar morphologies may overlap with the current assumed distribution of *Bryophryne*. Likewise, the northwestern limits of the distribution of *Psychrophrynella* overlap partially with the distribution of *Bryophryne* (e.g., *P. bagrecito, P. usurpator*).

Acknowledgments

Specimens for comparison were kindly loaned by D. Frost and D. Kizirian (AMNH), W. E. Duellman, L. Trueb and A. Campbell (KU), J. H. Córdova and C. Aguilar (MUSM), E. López (MUSA), P. Yanque and R. Orellana (MHNC). The Instituto Nacional de Recursos Naturales (INRENA) provided logistics and permits through the project "Evaluación de Flora y Fauna en la Puna del Santuario Nacional Megantoni". Asociación para la Conservación de la Cuenca Amazónica (ACCA) and Sociedad Zoológica de Frankfort (SZF) partially funded the expedition that resulted in the discovery of the new species (fieldwork was conducted by R. Gutiérrez, D. Huamán, A. Pari, C. Condori, and W. L. Delgado). Idea Wild provided field equipment and Smithsonian Conservation Biology Institute provided laboratory equipment. Research by J. C. Chaparro was supported by a grant from Javier Bustamante (Bakersfield Pediatrics) and family, Bakersfield, CA. Partial support was provided by projects CLG2008-04164 and CLG2011-30393 of the National Plan for Research, Development and Innovation of the Spanish Government (PI, I. De la Riva). Collecting activities were part of the project "Linea base del Sector Lacco-Santuario Nacional Megantoni-INRENA" (permit number 05 C/C-2008-INRENA-IANP-JSNM). We are grateful to W. E. Duellman and an anonymous reviewer for their constructive criticism.

References

Bazinet, A.L., Zwickl, D.J., Michael, P. & Cummings, P. (2014) A Gateway for Phylogenetic Analysis Powered by Grid Computing Featuring GARLI 2.0. *Systematic Biology*, 63, 812–818. http://dx.doi.org/10.1093/sysbio/syu031

Bossuyt, F. & Milinkovitch, M.C. (2000) Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proceedings of the National Academy of Sciences (USA)*, 97, 6585–6590. http://dx.doi.org/10.1073/pnas.97.12.6585

Canedo, C. & Haddad, C.F.B. (2012) Phylogenetic relationships within anuran clade Terrarana, with emphasis on the

placement of Brazilian Atlantic rainforest frogs genus *Ischnocnema* (Anura: Brachycephalidae). *Molecular Phylogenetics and Evolution*, 65, 610–620.
http://dx.doi.org/10.1016/j.ympev.2012.07.016

Chaparro, J.C., De la Riva, I., Padial, J.M., Ochoa, J.A. & Lehr, E. (2007) A new species of *Phrynopus* from Departamento Cusco, southern Peru (Anura: Brachycephalidae). *Zootaxa*, 1618, 61–68.

Crawford, A.J. (2003) Huge populations and old species of Costa Rican and Panamanian dirt frogs inferred from mitochondrial and nuclear gene sequences. *Molecular Ecology*, 12, 2525–2540.
http://dx.doi.org/10.1046/j.1365-294X.2003.01910.x

De la Riva, I. (2007) Bolivian frogs of the genus *Phrynopus*, with the description of twelve new species (Anura: Brachycephalidae). *Herpetological Monographs*, 21, 242–278.
http://dx.doi.org/10.1655/07-011.1

De la Riva, I. & Burrowes, P.A. (2014) A new species of *Psychrophrynella* (Anura: Craugastoridae) from the Cordillera Real, Department La Paz, Bolivia. *Zootaxa*, 3887 (4), 459–470.
http://dx.doi.org/10.11646/zootaxa.3887.4.4

Duellman, W.E. & Lehr, E. (2009) *Terrestrial–Breeding Frogs (Strabomantidae) in Peru*. Natur und Tier, Verlag GmbH, 382 pp.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D. & Kluge, A.G. (1996) Parsimony jackknifing outperforms neighbor–joining. *Cladistics*, 12, 99–124.
http://dx.doi.org/10.1111/j.1096-0031.1996.tb00196.x

Frost, D.R. (2015) Amphibian Species of the World: an Online Reference. Version 6.0 (Date of access). Electronic Database. American Museum of Natural History, New York, USA. Available from: http://research.amnh.org/herpetology/amphibia/index.html (accessed 30 June 2015)

Goloboff, P.A. (1996) Methods for faster parsimony analysis. *Cladistics*, 12, 199–220.
http://dx.doi.org/10.1111/j.1096-0031.1996.tb00009.x

Goloboff, P.A. (1999) Analyzing large data sets in reasonable times, solutions for composite optima. *Cladistics*, 15, 415–428.
http://dx.doi.org/10.1111/j.1096-0031.1999.tb00278.x

Goloboff, P.J., Farris, S. & Nixon, K. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, 24, 774–786.
http://dx.doi.org/10.1111/j.1096-0031.2008.00217.x

Hedges, S.B., Duellman, W.E. & Heinicke, M.P. (2008) New World direct developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. *Zootaxa,* 1737, 1–182.

Heinicke, M.P., Duellman, W.E. & Hedges, S.B. (2007) Major Caribbean and Central American frog faunas originated by ancient oceanic dispersal. *Proceedings of the National Academy of Sciences USA,* 24, 10092–10097.
http://dx.doi.org/10.1073/pnas.0611051104

Hillis, D. M., Moritz, C. & Mable, B.K. (1996) *Molecular Systematics. 2nd Edition*. Sinauer Associates Inc., Sunderland, Massachusetts, 655 pp.

Jungfer, K.H., Faivovich, J., Padial, J.M., Castroviejo-Fisher, S., Lyra, M., Berneck, B., Iglesias, P., Kok, P.J.R., MacCulloch, R.D., Trefaut-Rodrigues, M., Verdade, V.K., Torres-Gastello, C.P., Chaparro, J.C., Valdujo, P.H., Reichle, S., Moravec, J., Gvoždík, V., Gagliardi-Urrutia, Ernst, R., De la Riva, I., Means, D.B., Lima, A.P., Señaris, J.C., Wheeler, W.C. & Haddad, C.F.B. (2013) Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle. *Zoologica Scripta*, 42, 351–380.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005) MAFFT, a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059–3066.
http://dx.doi.org/10.1093/nar/gkf436

Lanfear, R., Calcott, B., Ho, S.Y. & Guindon, S. (2012) PartitionFinder, combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, 29, 1695–1701.
http://dx.doi.org/10.1093/molbev/mss020

Lehr, E. & Catenazzi, A. (2008) A new species of *Bryophryne* (Anura: Strabomantidae) from southern Peru. *Zootaxa,* 1784, 1–10.

Lehr, E. & Catenazzi, A. (2009) Three new species of *Bryophryne* (Anura: Strabomantidae) from the region of Cusco, Peru. *South American Journal of Herpetology*, 4, 125–138.
http://dx.doi.org/10.2994/057.004.0204

Lehr, E. and Catenazzi, A. (2010) Two new species of *Bryophryne* (Anura: Strabomantidae) from high elevations in southern Peru (Region of Cusco). *Herpetologica*, 66, 308–319.
http://dx.doi.org/10.1655/09-038.1

Lehr, E., & Oróz, A. (2012) Two new species of *Phrynopus* (Anura: Strabomantidae) from the Cordillera de Carpish in central Peru (Departamento de Huánuco). *Zootaxa*, 3512, 53–63.

Lehr, E., Fritzsch, G. & Müller, A. (2005) Analysis of Andes frogs (*Phrynopus*, Leptodactylidae, Anura) phylogeny based on 12S and 16S mitochondrial rDNA sequences. *Zoologica Scripta*, 6, 593–603.
http://dx.doi.org/10.1111/j.1463-6409.2005.00212.x

Lehr, E., Moravec, J. & Cusi, J.C. (2012) Two new species of *Phrynopus* (Anura, Strabomantidae) from high elevations in the Yanachaga-Chemillén National park in Peru (Departamento de Pasco). *ZooKeys*, 235, 51–71.

http://dx.doi.org/10.3897/zookeys.235.3885
Lynch, J.D. (1975). A review of the Andean leptodactylid frog genus *Phrynopus*. *Occasional Papers of the Museum of Natural History, University of Kansas*, 35, 1–51.
Mamani, L. & Malqui, S. (2014) A new species of *Phrynopus* (Anura: Craugastoridae) from the central Peruvian Andes. *Zootaxa*, 3838 (2), 207–214.
http://dx.doi.org/10.11646/zootaxa.3838.2.5
Padial, J. M., Castroviejo-Fisher, S. and De la Riva, I. 2009. The phylogenetic relationships of *Yunganastes* revisited (Anura: Terrarana). *Molecular Phylogenetics and Evolution*, 52, 911–915.
Padial, J.M., Chaparro, J.C., Castroviejo-Fisher, S., Guayasamín, J.M., Lehr, E., Delgado, A.J., Vaira, M., Teixeira, M., Aguayo, R. & De la Riva, I. (2012) A revision of species diversity in the Neotropical genus *Oreobates* (Anura: Strabomantidae), with the description of three new species from the Amazonian slopes of the Andes, and the proposal of candidate species. *American Museum Novitates*, 3752, 1–55.
http://dx.doi.org/10.1206/3752.2
Padial, J.M., Grant, T. & Frost, D.R. (2014). Molecular systematics of terraranas (Anura: Brachycephaloidea) with an assessment of the effects of alignment and optimality criteria. *Zootaxa*, 3825 (1), 1–132.
http://dx.doi.org/10.11646/zootaxa.3825.1.1
Pereyra, M.O., Cardozo, D., Baldo, J.L. & Baldo, D. (2014) Description and phylogenetic position of a new species of *Oreobates* (Anura: Craugastoridae) from northwestern Argentina. *Herpetologica*, 70, 211–227.
http://dx.doi.org/10.1655/HERPETOLOGICA-D-13-00072
Pyron, R.A. & Wiens, J.J. (2011) A large-scale phylogeny of Amphibia including over 2,800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution*, 61, 543–583.
http://dx.doi.org/10.1016/j.ympev.2011.06.012
Teixeira, M. Jr., Amaro, R.C., Recoder, R.S., de Sena, M.A. & Rodrigues, M.T. (2012) A relict new species of *Oreobates* (Anura, Strabomantidae) from the seasonally dry tropical forests of Minas Gerais, Brazil, and its implication to the biogeography of the genus and that of South American dry forests. *Zootaxa*, 3158, 37–52.
Wheeler, W.C. (1996) Optimization alignment, the end of multiple sequence alignment in phylogenetics? *Cladistics*, 12, 1–10.
Wheeler, W.C. (2003) Implied alignment, a synapomorphy-based multiple sequence alignment method and its use in cladogram search. *Cladistics*, 19, 261–268.
http://dx.doi.org/10.1111/j.1096-0031.2003.tb00369.x
Wheeler, W.C., Arango, C.P., Grant, T., Janies, D., Varón, A., Aagesen, L., Faivovich, J., D'Haese, C., Smith, W.L. & Giribet, G. (2006) *Dynamic Homology and Phylogenetic Systematics, A Unified Approach Using POY.* American Museum of Natural History, New York, 365 pp.
Wheeler, W.C., Lucaroni, N., Hong, L., Crowley, L.M. & Varón, A. (2015) POY version 5: phylogenetic analysis using dynamic homologies under multiple optimality criteria. *Cladistics,* 31, 189–196.
http://dx.doi.org/10.1080/10635150500234625
Wiens, J.J., Fetzner, J.W., Parkinson, C.L. & Reeder, T.W. (2005) Hylid frog phylogeny and sampling strategies for speciose clades. *Systematic Biology*, 54, 719–748.
http://dx.doi.org/10.1080/10635150500234625
Zwickl, D.J. (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation, The University of Texas at Austin. [unkown pagination]